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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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**(54) Title:** ENRICHED PLATELET WOUND HEALANT**(57) Abstract**

A wound healant composition comprising activated growth factors and ascorbic acid and/or at least one retinoid and/or at least one antibiotic, that facilitates the growth of new tissue.

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## ENRICHED PLATELET WOUND HEALANT

### BACKGROUND OF THE INVENTION

The invention disclosed herein generally relates to a composition of matter used in the treatment of wounds, a method of making same, and a method of using same.

There have been many different substances and methods developed in the past for treating wounds, depending upon the type and location and severity of the wound. A wound is generally defined as an injury to an area of the body. Although injury to the surface of the skin is the most well known type of wound, the surfaces of internal organs may also be wounded, such as during surgery or resulting from traumatic blows to the body surface in the vicinity of the internal organ.

Medical practice characterizes wounds as chronic or acute, according to the persistency and severity of the wound. A chronic wound is one that is prolonged or lingering, rather than promptly healed. An acute wound is one that occurs relatively quickly, and heals relatively quickly as well. Tissue wounds may have a wide spectrum of manifestations, as small as merely an abnormal microscopic tear or fissure in tissue (or a surface thereof), or as large as the abrasion or ablation of the skin covering a substantial portion of the body, such as in a burn victim. Acute wounds covering a large or movable surface are usually the most difficult to guard from infection, and to heal.

The invention described herein is primarily related to substances topically applied to the exterior surface of wounds upon the skin, although the invention described herein also has some applications for facilitating the healing of other wounds. The composition of matter described herein is especially suited to topical application to burn wounds and chronic lesions, such as ulcers on the feet of diabetics. However, the compositions of matter and the methods described herein are not limited solely to that topical application.

Wound healing is affected by the presence of various substances found in the blood and bodily fluids. The blood is the primary medium for delivering healing agents to the wound site, and for transporting foreign or harmful substances away from the wound. Whole blood is

primarily comprised of three main types of cells suspended in a protein rich solution known as plasma.

The three main cell types of whole blood are erythrocytes (a.k.a. red blood cells), leukocytes (a.k.a. white blood cells) and thrombocytes (a.k.a. platelets). The red blood cells are the iron-containing cells that facilitate the transport and transfer of oxygen to body tissue, and the removal of carbon dioxide. The white blood cells perform functions such as phagocytosis of foreign bodies and production of antibodies, primarily responsible for fighting infection and foreign substances within the blood or wound site. Platelets perform many functions such as plugging leaks in blood vessels and helping begin the process leading to the formation of a blood clot; platelets contain substances known as growth factors that facilitate the formation of new tissue.

Although there are several methods for separating whole blood into its various components, one of the most convenient and expeditious methods is accomplished by differentially centrifuging blood or some of its components (i.e., apheresis). In this way, the red and white blood cells and plasma may be separated out and returned to the donor's or patient's body, leaving the sequestered platelets in essentially concentrated form for use in wound healing techniques. From blood extracted from a patient, the platelets may thus be obtained and activated for use on the same patient; methods of using a patient's own blood are called "autologous" or "autogenic" donor methods. Methods using blood donated by one or more third parties for use by a patient are called "homologous" or "heterologous" donor methods, or collectively called "allogenic" methods.

One of the proteins suspended in plasma is fibrinogen, which reacts with substances released into (or attracted by) wound sites to produce sticky strands of fibrin. Such reactions result in the cross linking of the strands to form a mesh that holds and supports the deposit or growth of other tissue materials at the wound site.

The wound healing process is generally considered to occur in several stages, generally known as the healing cascade. After tissue injury, platelets are among the first cells to appear in the vicinity of the wound. Activation of a platelet by an agonist such as thrombin, or other agonists such as those listed elsewhere herein, leads to the release of granules from within the platelet. Such degranulation results in the release of proteins known as growth factors, concentrated in the alpha granules of platelets. Those released growth factors stimulate the

formation of new tissue; when applied to wounds, growth factors have been known to increase the rate of collagen laydown, vascular ingrowth, fibroblast proliferation and overall healing.

The release of a protein known as platelet-derived growth factor (PDGF) is a chemotactic for monocytes, neutrophils and fibroblasts into the wound, to begin the inflammatory stage of the healing process. During this time, monocytes secrete PDGF and another platelet protein known as transforming growth factor- $\beta$ 1, which recruits and activates fibroblasts, a precursor to fibrinogen, to begin the repair stage of the healing process. Subsequently, wound healing continues through the process of collagen remodeling within the wound.

The presence of growth factors promotes wound healing. The invention described herein increases the amount of growth factors in the wound, and thereby facilitates the promotion of the healing rate. This may be especially important in wounded patients who may lack sufficient circulation to facilitate the healing cascade. The invention described herein also facilitates the covering of the wound area with a substance that prevents infection caused by most bacteria; and to the extent that the wound treatment material is made from autologous blood or similar biological materials, the invention described herein reduces the risks associated with the use of treatment materials made from biological materials obtained from one or more third parties. Already known in the field are wound sealants made from biological materials obtained primarily from tissue other than blood platelets. For example, wound sealants include "fibrin glue," which often is essentially a mixture of coagulants (thrombin and calcium), concentrated fibrinogen and other coagulation proteins. In most applications, the primary roles of fibrin glue are to seal wound surfaces to prevent loss of blood and other body fluids after surgery, and to provide adhesion between adjacent tissue surfaces.

The production of fibrin glue often requires obtaining fibrinogen from blood through a process known as cryoprecipitation, including both freeze-thaw cycles and relatively lengthy centrifugation of plasma in controlled environments, to concentrate the fibrinogen in large enough amounts required for use; the precipitant thus obtained is often frozen before storage. These requirements make such materials unsuitable for application during the course of surgery; moreover, to the extent this process depends upon the use of autologous biological materials, using this process shortly before or during surgery may result in the loss of crucial bodily fluids during a time when the patient's body is badly in need of such fluids. By contrast, substantially

larger amounts of concentrated platelets can be more conveniently obtained within a matter of minutes from more recent methods of differential blood centrifugation not requiring freezing, without significant loss of bodily fluids.

To date, there has been much research concerning fibrin glue. This is considered to be a separate field from the present invention, primarily because fibrin glues typically contain cryoprecipitated proteins without platelets. The use of fibrin glue is discussed extensively in the scientific literature; for example, see the references cited in U.S. patent number 5,585,007 issued to Antanavich *et al* on December 17, 1996.

One method of differential centrifugation essentially allows separating the patient's own blood into at least three different components: packed erythrocytes (red blood cells), plasma and platelet concentrate. Platelet concentrate can be combined with a solution of either sodium or calcium mixed with thrombin ("calcified thrombin"), often to form a gelatinous composition of activated platelets that, when made with the necessary viscosity, can be utilized as a wound sealant. Such sealants typically set up into a hard mass covering the application site, thereby sealing the site. The initially sticky, gelatinous state usually hardens to serve the functions of (1) stopping the loss of blood and other bodily fluids, because it effectively acts as a patch; (2) sealing wounds against external contaminants; and (3) preventing traditional problems associated with the mere stitching of wounds.

Wound healing compositions including platelets have advantages over materials without platelets. One reason is that natural wound healing agents are released by the platelets. Further, the concentration of platelets likewise allows for a concentrated amount of wound healing factors. Additionally, to the extent that the wound healing composition is made from the biological materials of the patient, the risks associated with heterologous donors (such as disease, immunologic reactions, or the like) are eliminated.

The work surrounding the field of autologous platelet gel to date has focused on perioperative blood treatment - preventing loss of blood during or immediately following surgery. Normally, when a patient is on the operating table, the patient will lose large amounts of blood and other bodily fluids, depending upon the type of surgery involved. To counter this blood loss, the traditional approach is to infuse the patient with blood, which is usually donated from one or more third parties (or sometimes donated by the patient in anticipation of surgical needs). There exists many different types of methods for collecting blood that are normally used

in this type situation.

Because there is obviously an increased risk of disease, immunologic reaction, or other complications associated with procedures including heterologous blood donation, recent efforts have been made to use blood contemporaneously obtained from the patient during surgery. This blood can be fractionated and/or filtered, and subsequently re-infused into the patient, saving much time, expense, bodily fluids and avoiding normal risks discussed above.

It is from the perioperative blood treatment arena that the uses for autologous platelet gel were focused. The use of platelet gel on open wounds resulting from surgery have recently met great success. This particular use allows the patient to keep his/her own blood and also reduce costs.

The following patents are arguably related to the invention disclosed herein:

<u>Patent Number</u>	<u>Inventor</u>	<u>Date</u>
5,733,545	Hood	March 31, 1998
5,585,007	Antanavich, et al.	December 17, 1996
5,165,938	Knighton	November 24, 1992

The Hood patent claims a plasma-buffy coat concentrate comprising plasma, platelets (at a concentration of at least  $10^9$  cells/ml), fibrinogen (at a concentration of at least 5 mg/ml), and white blood cells (at a concentration of at least 3 times  $10^7$  cells/ml). Aside from the limitations imposed by said concentrations, the Hood invention fails to recognize the benefits of increased levels of vitamins, antibiotics and other substances. For instance, higher amounts of vitamin C is believed to prolong the viscosity and longevity of a gelatinous composition of fibrinous matter derived substantially from platelets. As another example, the Hood invention fails to recognize the benefits of including retinoids, such as vitamin A (retinol) and/or vitamin E. For instance, patients undergoing treatment including steroids often have immune systems that are suppressed, or otherwise non-responsive to stimuli; increased amounts of vitamin A are known to counteract that non-responsiveness, and thereby facilitate the promotion of wound healing. Similarly, increasing the level of vitamin E is believed to facilitate the promotion of wound healing.

The Knighton patent discloses the use of isolated growth factors combined with a biologically compatible carrier substance, after sequestration (and removal) of all platelet

membranes and plasma containing fibrin from the growth factor exudate prepared. Discarding such membranes essentially removes from the composition growth factors known to be concentrated in the membranes, and potential receptor sites for facilitating matrix formation. The method utilized in Knighton also requires a number of time consuming and labor intensive steps, including storage at a freezing temperature prior to use. The Knighton method also requires that wound treatments be repeated on a daily basis.

The Antanavich patent discloses a composition based primarily on plasma and, like Knighton, requires a biologically acceptable carrier for administering a plasma concentrate comprising platelets, fibrinogen and fibrinectin. The Antanavich composition is essentially a fibrin glue meant to have a high tensile strength (viscosity), sufficient to seal a wound.

The chemical reactions and cascades that normally happen when thrombin is added to the concentrated platelets are indeed complex. They are discussed in the scientific article by Reeder, et al, in Proceedings of the American Academy of Cardiovascular Perfusion, Vol. 14, January 1993. Adding a preservative, or healing promotion materials that do not detract from, substantially interfere with, or even destroy these different reactions is the crux of the invention disclosed herein.

One object of the invention is to provide a wound treatment material that is capable of quick and convenient production in the presence of the patient.

Another object of the invention is to provide a wound treatment material that facilitates the promotion of wound healing.

Another object is to provide a wound treatment material that facilitates the prevention of wound infection.

Another object is to provide a method of making a wound treatment material satisfying the objectives expressed, implied or inherent herein.

Another object is to provide a method of using a wound treatment material satisfying the objectives expressed, implied or inherent herein.

### **SUMMARY OF THE INVENTION**

In most general terms, the invention described herein expands the uses for concentrated platelet materials, especially those in gel form, by improving the speed and convenience of making the composition; the invention described herein also improves the performance of the

concentrated platelet composition, by making it more useable for applications over longer periods of time, and by enhancing the wound healing and infection fighting properties. For autologous platelet gel to be more useful, the gelatinous state must be capable of remaining stable for a reasonable period of time. One aspect of the present invention is to add a preservative to the platelet gel.

Another aspect of the present invention involves adding one or more antibiotic substance at one or more times during the processing period so that the resulting concentrated platelet composition contains either one or a variety of the antibiotics. The use of an antibiotic in concentrated platelet compositions that enhances the complex healing cascade is indeed novel. The invention disclosed herein involves adding such substances in a manner that does not detract from, substantially interfere with, or even destroy these different reactions, pH balances and potency.

Another aspect of the present invention involves adding one or more vitamins known to promote wound healing.

The method of making the invention in gel form described herein includes mixing at least one of the described additives with the plasma-poor concentrated platelets, a sufficient time before the addition of calcified thrombin (or other preferably-calcified agonist) to allow the desired dispersion of such additive(s) in such composition before gelation prevents further dispersion.

#### **DETAILED DESCRIPTION OF THE INVENTION**

Before the present invention is described in detail, it is to be understood that the invention is not limited to the particular configurations, process steps and materials expressly disclosed herein; the invention includes those that are implicit or inherent in the disclosures set forth herein, and all legal equivalents of any element(s) or limitation(s) thereof. As an example, the biological materials specified herein may originate from a patient to be treated, from a single third party, or from a plurality of third parties; moreover, said third parties may be of the same species as the patient, or of another species, so long as the wound treatment material derived from such biological materials is biocompatible with the patient. As another example, when the invention calls for a particular substance, it is sufficient to use any form of that substance having the characteristic(s) needed to satisfy the stated need; for instance, unless the context indicates

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otherwise, a need for growth factors may be satisfied by providing isolated growth factors or those that are included in platelets or other types of cells, and/or combinations thereof. Similarly, the process deployed to obtain the growth factors may be any process that satisfactorily does so, regardless of whether it includes centrifugation.

It is also to be understood that the terminology used herein is not intended to be limiting, since the scope of the present invention will be limited only by the claims and equivalents thereof. Also, as used herein, the singular forms include the plurals, and vice versa, unless the context indicates otherwise.

For the sake of simplicity and to give the claims of this patent application the broadest interpretation and construction possible, the following definitions will apply:

- (a) The phrase *blood collecting* or *blood extraction* (or similar phrase) includes techniques, materials and apparatus known in the field, such as (for example) inclusion of anticoagulation materials, the use of blood drawing and infusion apparatus.
- (b) The phrase *growth factor* means any material(s) promoting growth of tissue.
- (c) The term *thrombin* may include calcified thrombin, in particular, about 5,000 units of thrombin per ml of aqueous calcium chloride.
- (d) The term *viscosity* means those characteristics of the specified material(s) determining the degree of gelation, such as (for example) the firmness or hardness of the material, or the degree to which the material resists flowing like a fluid.

Also for the sake of simplicity, the conjunctive "and" may also be taken to include the disjunctive "or," and vice versa, whenever necessary to give the claims of this patent application the broadest interpretation and construction possible. Likewise, when the plural form is used it may be taken to include the singular form and vice versa.

In most general terms, the invention includes a wound healant composition comprising activated growth factors and ascorbic acid. In the prevalent version of the invention, said growth factors are included within platelets. The body produces many substances generally known as growth factors, and the growth factors of the present invention are selected from the group consisting of platelet-derived growth factor (PDGF), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), platelet-derived epidermal growth factor

(PDEGF), platelet factor 4 (PF-4), transforming growth factor  $\beta$  (TGF-B), acidic fibroblast growth factor (FGF-A), basic fibroblast growth factor (FGF-B), transforming growth factor  $\alpha$  (TGF-A), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2),  $\beta$  thromboglobulin-related proteins (BTG), thrombospondin (TSP), fibronectin, von Wallinbrand's factor (vWF), fibropeptide A, fibrinogen, albumin, plasminogen activator inhibitor 1 (PAI-1), osteonectin, regulated upon activation normal T cell expressed and presumably secreted (RANTES), gro- $\alpha$ , vitronectin, fibrin D-dimer, factor V, antithrombin III, immunoglobulin-G (IgG), immunoglobulin-M (IgM), immunoglobulin-A (IgA),  $\alpha$ 2-macroglobulin, angiogenin, Fg-D, elastase, keratinocyte growth factor (KGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), tumor necrosis factor (TNF), fibroblast growth factor (FGF) and interleukin-1 (IL-1), and combinations thereof.

Activation of growth factors may occur in a variety of manners, by a variety of substances known as activators. In the invention described herein, said activation results from the inclusion of an activator selected from the group consisting of thrombin, collagen, serotonin, adenosine diphosphate (ADP) and acetylcholine (ACH), and combinations thereof. In a particular version of the invention, said growth factors are included within concentrated platelets, and said activation results from the inclusion of thrombin.

The invention is not limited to autologous biological materials, such as where said concentrated platelets are obtained from the wounded's own biological material. The invention encompasses the use of biological materials obtained from one or more third parties, that need not be of the same species as the patient whose wound is being treated with the wound healant composition described herein unless bioincompatibility would result from the use of such third party biological materials.

In one general version of the invention, the wound healant composition includes concentrated platelets, thrombin and ascorbic acid. Since the admixture of thrombin or other agonists will activate growth factors, the thrombin (or other agonists/activators) should usually be the last substance to be mixed immediately before it is desired that the gelatinous state be set up.

Another version of the invention includes the inclusion of at least one retinoid in the admixture, in addition to or in substitution of the ascorbic acid. Although the wound healant composition could include a combination of retinoids, one version of the invention merely

substitutes vitamin A in addition to or in substitution of the ascorbic acid. Vitamin A has been known to counteract a side effect of some treatments using steroids, namely, the depressed reactivity of the body immune system to stimuli. In another version, said retinoid is vitamin E, known to facilitate healing.

Although the wound healant composition could include a combination of antibiotics, one version of the invention merely substitutes at least one antibiotic in addition to or in substitution of the ascorbic acid. Since many wound sites are either already infected with bacteria or are susceptible to such infection, it is desirable that a wound healant composition be capable of either killing bacteria or preventing the mobility or reproduction of bacteria. The invention described herein includes a wound healant composition comprising concentrated platelets, thrombin and at least one antibiotic. In particular, the invention includes a wound healant wherein said antibiotic is bacteriocidal to at least *Pseudomonas* and *Klebsella* bacteria, which are prevalent at wound sites and difficult to guard against. Alternatively, said antibiotic is selected from the group consisting of a neosporin, vancomycin and gentamycin, and combinations thereof.

As indicated above, the invention may include a wound healant composition comprising concentrated platelets, thrombin, ascorbic acid, at least one retinoid and at least one antibiotic bacteriocidal to *Pseudomonas* and *Klebsella* bacteria.

Aside from the wound healant substance to be applied to wound sites, the invention further includes a method of making a wound healant composition. One manner of making the plasma-poor concentrated platelets of the present invention is to collect about 450 ml of whole blood in anticoagulant (such as sodium citrate or any similar anticoagulant known in the field). That blood is then centrifuged at at least one speed in the range of between about 2,000 rpms and 3,000 rpms (preferably about 2,400 rpms) for a duration of between about 15 minutes and 25 minutes (preferably about 20 minutes) to separate out a band (or similar grouping) of: (a) plasma and most white blood cells; (b) platelets (and incidental white blood cells); and (c) red blood cells. The platelet portion may then be re-centrifuged to further separate out plasma (and incidental white blood cells); said re-centrifuging may be at at least one speed in the range of between about 4,000 rpms and 5,600 rpms (preferably about 4,800 rpms) for a duration of between about 5 minutes and 10 minutes (preferably about 7½ minutes).

The final yield is about 40 to 50 ml of plasma-poor concentrated platelets (in trace or

incidental amounts of residual plasma and white blood cells). Both the plasma/leukocyte portion and the red blood cell portion may be reinfused back into the patient. The plasma-poor concentrated platelets may then be activated by a mixture of thrombin and (preferably) calcium. Preferably, a solution comprising 5,000 units of thrombin per ml of calcium chloride solution will result; since blood anticoagulants typically tie up blood calcium to prevent the clotting cascade from occurring, calcified thrombin is used to re-supply the plasma-poor concentrated platelets with more calcium to facilitate the clotting cascade at the wound site. Depending upon the relative concentrations of the ingredients, the resulting mixture may be either a liquid, or it may set up as a hard material or (preferably) as a gel having a viscosity dependant upon the relative amounts of thrombin and platelets; the relative concentrations of calcified thrombin to platelets determines how quickly the composition sets up, and how hard it will eventually be. Some mixtures will yield a composition that will set up in a gel in several seconds, whereas some mixtures will yield a composition that takes several minutes to set up in a gel.

Regardless of the amount of set up time, the present invention includes a preservative that allows the gel to retain its viscosity for a longer duration. For example, ascorbic acid is believed to preserve the longevity of the gel viscosity. Another method of making the wound healing composition includes the steps of mixing activated growth factors with ascorbic acid. Said activated growth factors may be obtained in a variety of ways, such as by the steps of sequestering concentrated platelets from blood and mixing thrombin with said platelets. Said ascorbic acid should be in sufficient amount to enhance the preservation of the gelatinous state of the final wound healing composition, and said thrombin should be in sufficient amount to facilitate gelation having the desired level of viscosity while sufficiently activating growth factors present in the composition and the wound.

In one preferred version of the composition, about 1 ml of ascorbic acid is mixed with about 8 ml of concentrated platelets, then about 1 ml of calcified thrombin is mixed into that 9 ml admixture. However, other ratios of concentrated platelets:ascorbic acid:thrombin may be useful, depending upon the desired amount of healing agents, gelation time, gel viscosity and longevity. For instance, if a gel is desired that will set up with a harder viscosity, more thrombin should be used.

Another method of making the composition allows the extraction of blood, sequestering of plasma-poor concentrated platelets, mixing of additives and return of unused blood

components to the patient, all in about 20 to 30 minutes and by making only one puncture in the patient. Approximately 125 ml of blood is extracted from a patient, with the blood drawing apparatus optionally remaining in place connected to the patient (for later use in returning unused blood components to the patient). That blood is transferred to a Latham bowl and, using a centrifuge such as is manufactured by Haemonetics, Inc., centrifuged at about 4,800 rpms until a band (or similar grouping) of plasma forms at the upper periphery (about 5 to 15 minutes), and a band (or similar grouping) of red blood cells forms at the bottom of the bowl; the center is comprised of plasma-poor concentrated platelets. The plasma band is removed for return to the patient, and the remaining blood components are again centrifuged at that speed (and sufficient duration), further removing plasma and white blood cells from the plasma-poor concentrated platelets. The plasma-poor concentrated platelets are then removed for mixing with the other additives described herein (thrombin, ascorbic acid and/or retanoids).

The method of making a wound healant may further include, prior to or contemporaneous with mixing said thrombin, mixing at least one of the aforementioned retinoids in sufficient amount(s) to further enhance wound healing. In one version, 1 ml of aqueous vitamin A and vitamin E solution is added to 8 ml of concentrated platelets and 1 ml of ascorbic acid, before mixing in 1 ml of thrombin.

Alternatively, said method may include, prior to or contemporaneous with mixing said thrombin, mixing at least one of the aforementioned antibiotics in sufficient amount(s) to reduce infection by bacteria.

Besides a method of making a wound healant composition, the invention described herein may also include a method of treating a wound, comprising the steps of applying a sufficient amount of a composition of matter comprising growth factors and ascorbic acid to enhance healing of the wound. Said method of treating a wound may include the use of any of the compositions described herein; it may also include the use of any composition made by any of the methods described herein.

Once applied to a wound, the composition may remain on the wound for as long as 5 days, and perhaps longer depending upon the circumstances such as the location of the wound and other wound characteristics. Although the composition and method described herein are especially useful for the treatment of chronic wounds, they may also be useful in the treatment of acute wounds.

## I claim:

1. A wound healant composition comprising activated growth factors and ascorbic acid.
2. A wound healant described in claim 1, wherein said growth factors originate in platelets.
3. A wound healant described in claim 1, wherein said growth factors are selected from the group consisting of platelet-derived growth factor (PDGF), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), platelet-derived epidermal growth factor (PDEGF), platelet factor 4 (PF-4), transforming growth factor  $\beta$  (TGF- $\beta$ ), acidic fibroblast growth factor (FGF-A), basic fibroblast growth factor (FGF-B), transforming growth factor  $\alpha$  (TGF-A), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2),  $\beta$  thromboglobulin-related proteins (BTG), thrombospondin (TSP), fibronectin, von Wallinbrand's factor (vWF), fibropeptide A, fibrinogen, albumin, plasminogen activator inhibitor 1 (PAI-1), osteonectin, regulated upon activation normal T cell expressed and presumably secreted (RANTES), gro- $\alpha$ , vitronectin, fibrin D-dimer, factor V, antithrombin III, immunoglobulin-G (IgG), immunoglobulin-M (IgM), immunoglobulin-A (IgA),  $\alpha$ 2-macroglobulin, angiogenin, Fg-D, elastase, keratinocyte growth factor (KGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), tumor necrosis factor (TNF), fibroblast growth factor (FGF) and interleukin-1 (IL-1), and combinations thereof.
4. A wound healant described in claim 1, wherein said activation results from the inclusion of an agonist selected from the group consisting of thrombin, collagen, serotonin, adenosine diphosphate (ADP) and acetylcholine (ACH), and combinations thereof.
5. A wound healant described in claim 1, wherein said growth factors are included within concentrated platelets, and said activation results from the inclusion of thrombin.
6. A wound healant described in claim 5, wherein said concentrated platelets are

obtained from the wounded's own biological material.

7. A wound healant composition comprising concentrated platelets, ascorbic acid and thrombin.
8. A wound healant composition comprising concentrated platelets, at least one retinoid and thrombin.
9. A wound healant composition described in claim 8, wherein said retinoid is vitamin A.
10. A wound healant composition described in claim 8, wherein said retinoid is vitamin E.
11. A wound healant composition comprising concentrated platelets, at least one antibiotic and thrombin.
12. A wound healant described in claim 11, wherein said antibiotic is bacteriocidal to at least *Pseudomonas* and *Klebsella* bacteria.
13. A wound healant described in claim 11, wherein said antibiotic is selected from the group consisting of a neosporin, vancomycin and gentamycin, and combinations thereof.
14. A wound healant composition comprising concentrated platelets, ascorbic acid, at least one retinoid, at least one antibiotic bacteriocidal to at least *Pseudomonas* and *Klebsella* and thrombin.
15. A wound healant composition comprising about 8 ml of concentrated platelets, about 1 ml of ascorbic acid and about 1 ml of thrombin.
16. A wound healant composition as described in claim 15, further comprising

about 1 ml of vitamin A and vitamin E.

17. A method of making a wound healant composition, comprising the steps of mixing activated growth factors with ascorbic acid.
18. A method of making a wound healant described in claim 17, wherein said activated growth factors are obtained by the steps of sequestering concentrated platelets and mixing thrombin with said platelets.
19. A method of making a wound healant described in claim 18, said sequestering comprising the steps of separating the platelets from the other blood components by centrifuging blood at at least one speed in the range of between about 2,000 rpms and about 3,000 rpms for a duration in the range of between about 15 minutes and about 25 minutes, then further concentrating said platelets by centrifuging is at at least one speed in the range of between about 4,000 rpms and about 5,600 rpms for a duration in the range of between about 5 minutes and about 10 minutes, and mixing ascorbic acid and then thrombin with said concentrated platelets in sufficient amounts to result in gelation having a desired level of viscosity and longevity.
20. A method of making a wound healant described in claim 19, wherein:  
for first separating said platelets, said centrifuge speed is about 2,400 rpms and said centrifugation duration is about 20 minutes;  
for further concentrating said platelets, said centrifuge speed is about 4,800 rpms and said centrifugation duration is about 7½ minutes; and  
about 1 ml of said ascorbic acid is mixed in about 8 ml of said concentrated platelets.
21. A method of making a wound healant described in claim 20 wherein said thrombin amount is about 5,000 units in about 1.0 ml of aqueous calcium chloride solution.
22. A method of making a wound healant described in claim 17, said method

about 1 ml of vitamin A and vitamin E.

17. A method of making a wound healant composition, comprising the steps of mixing activated growth factors with ascorbic acid.
18. A method of making a wound healant described in claim 17, wherein said activated growth factors are obtained by the steps of sequestering concentrated platelets and mixing thrombin with said platelets.
19. A method of making a wound healant described in claim 18, said sequestering comprising the steps of separating the platelets from the other blood components by centrifuging blood at at least one speed in the range of between about 2,000 rpms and about 3,000 rpms for a duration in the range of between about 15 minutes and about 25 minutes, then further concentrating said platelets by centrifuging is at at least one speed in the range of between about 4,000 rpms and about 5,600 rpms for a duration in the range of between about 5 minutes and about 10 minutes, and mixing ascorbic acid and then thrombin with said concentrated platelets in sufficient amounts to result in gelation having a desired level of viscosity and longevity.
20. A method of making a wound healant described in claim 19, wherein:  
for first separating said platelets, said centrifuge speed is about 2,400 rpms and said centrifugation duration is about 20 minutes;  
for further concentrating said platelets, said centrifuge speed is about 4,800 rpms and said centrifugation duration is about 7½ minutes; and  
about 1 ml of said ascorbic acid is mixed in about 8 ml of said concentrated platelets.
21. A method of making a wound healant described in claim 20 wherein said thrombin amount is about 5,000 units in about 1.0 ml of aqueous calcium chloride solution.
22. A method of making a wound healant described in claim 17, said method

an essentially intermediate grouping comprised of concentrated platelets therebetween; removing said plasma band; centrifuging said remaining blood components at said speed and sufficient duration; removing said concentrated platelets; and mixing said concentrated platelets with thrombin.

30. A method of making a wound healant composition described in claim 29, wherein: approximately 125 ml of blood is extracted from a patient; said blood is transferred to a Latham bowl and centrifuged at about 4,800 rpms about 10 minutes until said band of plasma forms at the upper periphery, said band of red blood cells forms at the bottom of said bowl, and said essentially intermediate grouping comprised of concentrated platelets forms therebetween; and said second centrifuging or said remaining blood components is at said speed and duration.
31. A method of treating a wound, comprising the steps of applying a sufficient amount of a composition of matter comprising activated growth factors and ascorbic acid to enhance healing of the wound.
32. A method of treating a wound described in claim 31, wherein said growth factors are derived from platelets.
33. A method of treating a wound described in claim 31, wherein said growth factors are selected from the group consisting of platelet-derived growth factor (PDGF), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), platelet-derived epidermal growth factor (PDEGF), platelet factor 4 (PF-4), transforming growth factor  $\beta$  (TGF- $\beta$ ), acidic fibroblast growth factor (FGF-A), basic fibroblast growth factor (FGF-B), transforming growth factor  $\alpha$  (TGF-A), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2),  $\beta$  thromboglobulin-related proteins (BTG), thrombospondin (TSP), fibronectin, von Wallenbrand's factor (vWF), fibropeptide A, fibrinogen, albumin, plasminogen activator inhibitor 1 (PAI-1), osteonectin, regulated upon activation normal T cell expressed and presumably secreted (RANTES), gro- $\alpha$ , vitronectin, fibrin D-dimer, factor V, antithrombin III,

immunoglobulin-G (IgG), immunoglobulin-M (IgM), immunoglobulin-A (IgA),  $\alpha$ 2-macroglobulin, angiogenin, Fg-D, elastase, keratinocyte growth factor (KGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), tumor necrosis factor (TNF), fibroblast growth factor (FGF) and interleukin-1 (IL-1), and combinations thereof.

34. A method of treating a wound described in claim 31, wherein said activation results from the inclusion of an agonist selected from the group consisting of thrombin, collagen, serotonin, ADP and acetylcholine (ACH), and combinations thereof.
35. A method of treating a wound described in claim 31, wherein said growth factors are derived from concentrated platelets, and said activation results from the inclusion of thrombin.
36. A method of treating a wound described in claim 31, wherein said concentrated platelets are obtained from the wounded's own biological material.
37. A method of treating a wound described in claim 31, said wound healant composition comprising concentrated platelets, ascorbic acid and thrombin.
38. A method of treating a wound described in claim 31, said wound healant composition comprising concentrated platelets and thrombin, and further comprising at least one retinoid.
39. A method of treating a wound described in claim 38, wherein said retinoid is vitamin A.
40. A method of treating a wound described in claim 38, wherein said retinoid is vitamin E.
41. A method of treating a wound described in claim 31, said wound healant

composition comprising concentrated platelets and thrombin, and further comprising at least one antibiotic.

42. A method of treating a wound described in claim 41, wherein said antibiotic is bacteriocidal to at least *Pseudomonas* and *Klebsella* bacteria.
43. A method of treating a wound described in claim 41, wherein said antibiotic is selected from the group consisting of a neosporin, vancomycin and gentamycin, and combinations thereof.
44. A method of treating a wound described in claim 31, said wound healant composition comprising concentrated platelets, ascorbic acid, at least one retinoid, at least one antibiotic bacteriocidal to *Pseudomonas* and *Klebsella* bacteria, and thrombin.

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : A01N 63/00; A61K 35/14, 35/16, 38/48

US CL : 424/93.72, 94.64, 529, 530, 532

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.72, 94.64, 529, 530, 532

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

USPATFULL- wound healing compositions containing platelets, activating factors, e.g., thrombin, antibiotics and vitamins herein.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,733,545 A (HOOD, III) 31 March 1998, entire document, especially columns 18-20.	1-44
Y	US 5,585,007 A (ANTANAVICH et al.) 17 December 1996, entire document, especially columns 25-28.	1-44
Y	US 5,165,938 A (KNIGHTON) 24 November 1992, entire document, especially column 6.	1-44
Y	US 5,674,912 A (MARTIN) 07 October 1997, abstract and column 13.	1-44



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

29 MARCH 1999

Date of mailing of the international search report

22 APR 1999

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